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Proton NMR transverse relaxation measurements to study water dynamic states and age-related changes in Mozzarella di Bufala Campana cheese

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Abstract

Proton transverse magnetization decay curves of Mozzarella di Bufala Campana samples were measured and analysed for the presence in the sample of four components, namely "serum water", "entrapped water", "junction zone water" and "fat", characterised by different transverse relaxation times, T_2. T_2 values were interpreted on the basis of the diffusive and chemical exchange model that allowed information to be obtained on the size of diffusive domains and dynamics of the water molecules. Furthermore, T_2 values were measured as a function of the aging time of Mozzarella samples, as produced by different cheese-makers. A decrease of "serum water" T_2 value with aging time was found and this may be used to monitor the evolution of Mozzarella samples up to seven days after manufacture.

Keywords: Mozzarella; Low-resolution NMR; Proton transverse relaxation time; Aging

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1. Introduction

Mozzarella di Bufala Campana cheese is among the most highly valued "pasta filata" (fresh and stretched curd) cheeses, obtained only from Buffalo (*Bubalus bubalus*) milk in Southern Italy, as certified by the European Union with the protected designation of origin (PDO) trademark. It is an unripened cheese, having a soft consistency, with a fresh and slightly acid taste and a pleasant aroma. The Mozzarella di Bufala Campana cheese has a porcelain white colour, its surface is smooth, bright and humid, and there is an extremely thin rind. The pasta has a white colour and a slightly elastic consistency, it appears to be composed of many layers with rare small cavities ("occhiature"). The Mozzarella di Bufala is high in moisture (55–60%) and in fat (24–25% on a dry matter basis, INRAN, 2003), it is very rich in whey and is normally stored at 4–7 °C in packages containing liquid (diluted whey, water from the stretcher-moulder or aqueous solution of sodium chloride). Generally, Mozzarella di Bufala is kept submerged in the preservation liquid for 3 or 4 days at a temperature of about 8–15 °C without loosing its characteristics. Mozzarella cheese is a very complex system and its water distribution differs from most kinds of cheese due to micro-structural differences resulting from the curd stretching process, which creates an elastic network of oriented parallel protein fibres (Kuo, Gunasekaran, Johnson, & Chen, 2001).

In a previous study (Gianferri, Maioli, Delfini, & Brosio, 2007), we have used an NMR protocol, based on both low-resolution and high-resolution NMR techniques, purposely to characterise Mozzarella di Bufala Campana cheese. High-resolution NMR was employed to determine the "chemical fingerprint" of the cheese complex matrix, i.e. the profile of water-soluble low molecular weight metabolites extracted from Mozzarella, while low-resolution NMR was used to investigate the most abundant

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components in Mozzarella – water and lipids – by evaluating the decay rates and amplitudes of the NMR signal. As discussed (Gianferri et al., 2007), proton transverse magnetization relaxation curves for Mozzarella cheese showed multi-exponential behaviour because of the presence of different components, water and lipids, as well as of different structural elements in the sample. The analysis of the transverse magnetization decay curve, in fact, evidenced the presence of four components, characterised by different T_2 values and amplitudes, ascribed to "serum water" – water accumulated in protein fibre channels –, "entrapped water" – water inside casein gel network –, "junction zone water" – water molecules that could be seen as an integral part of the protein structure – and fat molecules. In this paper, relaxation data have been discussed according to the model of diffusive and chemical exchange (Belton & Hills, 1987; Belton, Hills, & Raimbaud, 1988; Hills, Wright, & Belton, 1989), which allowed a new interpretation of proton NMR relaxation of water in foods and heterogeneous systems (Hills, Takacs, & Belton, 1990; Hills, Belton, & Quantin, 1993; Raffo, Gianferri, Barbieri, & Brosio, 2005). Without recourse to concepts such as "bound water" or multiple site occupancy, in fact, the observations that water transverse relaxation decay in heterogeneous systems is often multi-exponential, and that water relaxation rate is always faster than the corresponding relaxation rate in bulk water, can be quantitatively rationalized. In this paper, we show that a systematic approach to proton NMR relaxation, based on the understanding of the effects of the diffusive and chemical exchange, can provide valuable information about water dynamic state and morphology of Mozzarella cheese samples, thus allowing a thorough insight into the cheese physical structure and allowing NMR dynamic parameters to be related to the evolution of samples. Furthermore, we have studied Mozzarella di Bufala Campana samples of different ages from different cheese-makers, in order to demonstrate the possibility of using NMR relaxation data to evaluate Mozzarella aging.

2. Materials and methods

2.1. Cheese sample

Loaves of about 250 g of freshly prepared (day 1) Mozzarella di Bufala Campana (PDO) cheese were purchased from a commercial warehouse. They were produced by several artisan cheese-makers, all certified by "Mozzarella di Bufala Campana" consortium: Caseificio Fratelli Francia (Latina – Italy) – "Francia"; Caseificio Azienda Agricola Morese (Salerno – Italy) – "Taverna Penta"; Caseificio Cooperativa Allevatori di Bufale di Paestum (Salerno – Italy) – "Rivabianca"; Caseificio La Baronia (Caserta – Italy) – "La Baronia" and Caseificio Sorì Italia (Caserta – Italy) – "Sorì".

The cheese-makers declared shelf-lives of 10, 10, 6, 10, and 14 days for "Francia", "Taverna Penta", "Rivabianca", "La Baronia", and "Sorì" Mozzarella di Bufala Campana, respectively.

Mozzarella di Bufala Campana "Francia" samples were measured in triplicate by sampling three different loaves.

To explore the water relaxation behaviour associated with the age-related changes, our measurements began when Mozzarella cheese samples were at day 1 after manufacturing (fresh Mozzarella) and lasted for the following 14 days. Each cheese loaf was individually packaged in its conservation liquid and stored in a cold chamber at 8 ± 2 °C until tested.

2.2. Sample preparation

For low-resolution NMR measurements, four samples were prepared by cutting cylindrical-shaped pieces (diameter 8 mm and height 10 mm) out of the central part of the Mozzarella, at a distance of ca. 3 cm from the surface. The different aliquots were weighed (approximately 350 mg), then transferred into a NMR tube (outside diameter 10 mm). To prevent water evaporation, a teflon plug was inserted and the tube was then sealed by a laboratory film.

Furthermore, approximately 0.8 ml of conservation liquid was transferred into a NMR tube (outside diameter 10 mm), then a teflon plug was inserted and the tube sealed by a laboratory film.

2.3. NMR measurements

Measurements were performed with a Minispec mq 20 pulsed NMR spectrometer (Bruker Spectrospin Company, Milano, Italy), with an operating frequency of 20 MHz for protons (magnetic field strength: 0.47 T). The NMR spectrometer was equipped with an external thermostat (Julabo F25, Julabo Labortechnik GmbH, Seelbach, Germany) to maintain the desired temperature conditions. Before, the NMR measurements, the tube was kept in the NMR probe as long as needed for thermal equilibration (ca. 15 min). The measurements were taken at the storage temperature of Mozzarella cheese, i.e. 8 °C (±0.8 °C).

The transverse relaxation curves were measured by the Carr–Purcell–Meiboom–Gill (CPMG) sequence (Meiboom & Gill, 1958) with pulse spacing (τ_{cp}) between two following pulses of 0.04 ms. The first 200 data points of relaxation curve of Mozzarella samples were acquired after each echo signal (to detect the shortest T_2 value), then 6000 data points were acquired after each 10th echo (to detect the longer T_2 values) as an average of 16 repetitions, and with a recycle delay (RD) of 10 s. On the other hand, 6000 data points were acquired after each 10th echo as an average of 16 repetitions, and with a RD of 10 s to measure the relaxation time of conservation liquid samples.

The relaxation decay curves were fitted to exponential decays using Eq. (1)

$$A(\tau) = \sum_i A_{(i)} \exp(-\tau/T_{2(i)}) + L_0 \tag{1}$$

where $A(\tau)$ is the echo amplitude at the time τ, $T_{2(i)}$ and $A_{(i)}$ are the spin–spin relaxation time and amplitude, respectively, of component i, and the constant L_0 represents the decay curve noise. Mozzarella and conservation liquid samples were analysed in quadruplicate and the means and standard deviations were calculated.

3. Results and discussion

The NMR relaxation in heterogeneous systems, such as cheese, is affected by the food network structure because each given proton is influenced by its surroundings. This yields NMR very rich in information about the water mobility compared to other spectroscopic techniques. Therefore, NMR can provide information that cannot be gathered anywhere else (Karoui & De Baerdemaeker, 2007). However, the interpretation of T_2 curves is not completely unambiguous because it is affected by experimental conditions. The CPMG parameter setting and relaxation decay curves analysis is crucial for obtaining valuable results. The experimental strategies used to optimise the analysis of relaxation curves have been discussed in a previous paper (Gianferri et al., 2007).

The proton transverse magnetization relaxation curves of Mozzarella di Bufala Campana cheese show multi-exponential behaviour because of the presence of four components, characterised by different T_2 values and amplitudes. The results obtained for Mozzarella di Bufala Campana cheese by different artisan cheese-makers, as sampled at day 1 after manufacture, are summarised in Table 1.

According to the interpretation discussed in the previous paper (Gianferri et al., 2007), with reference to the data shown in the first row of Table 1 (Mozzarella di Bufala Campana "Francia"), the slowly relaxing component, $T_{2,\mathrm{sw}} = 951 \pm 36$ ms, was ascribed to water molecules remote from the casein matrix, whose motion is slightly affected by the presence of protein. This is the water accumulated in the large open channels formed by casein micelles aggregation in fibres ("serum water") with essentially the same properties as water in a solution. The fast relaxing component, $T_{2,\mathrm{jw}} = 7.3 \pm 0.3$ ms, was attributed to water molecules trapped within casein junction zones ("junction zone water"). This water can be seen as an integral part of the protein structure, trapped within the casein matrix in interstitial form. Its transverse relaxation time is essentially dominated by a fast chemical exchange with protein-exchangeable protons (–OH, –COOH, –NH, –SH), which have a shorter T_2 than has water. The component with a transverse relaxation time of $T_{2,\mathrm{ew}} = 47 \pm 3$ ms can be ascribed to water molecules inside meshes of the gel-like network made by casein molecules ("entrapped water"). This water can interact with the protein structure by diffusion from the bulk to biopolymer interface, and its T_2 value is controlled by diffusive exchange. Finally, the component whose relaxation time is $T_{2,\mathrm{f}} = 160 \pm 15$ ms, can be ascribed to protons of the lipid phase of Mozzarella cheese ("fat"). This assignment was made on the basis of the percentage of this component (20 ± 3%), which corresponds to the lipid content in the water plus fat phase of Mozzarella cheese samples (INRAN, 2003).

A discrepancy between the T_2 and percentage values of the four components obtained in this paper and the previous one could appear. However, it has to be pointed out that Mozzarella samples measured in the previous paper (Gianferri et al., 2007), as reported in Materials and methods, were drained on Whatman paper, before transferring into an NMR tube. So that the measured samples are physically different and the T_2 and percentage values cannot be compared.

Let us now discuss proton NMR relaxation data on the basis of the chemical and diffusive exchange model (Belton & Hills, 1987; Belton et al., 1988; Hills et al., 1989), that allows valuable information about water dynamics and morphology of Mozzarella cheese sample to be obtained.

3.1. Water relaxation measurements in fresh Mozzarella di Bufala Campana cheese

First, the observation in the casein gel-like network of two relaxation components, "junction zone water" ($T_{2,\mathrm{jw}}^{\mathrm{day1}} = 7.3 \pm 0.3$ ms) and "entrapped water" ($T_{2,\mathrm{ew}}^{\mathrm{day1}} = 47 \pm 3$ ms), is consistent with a regime of a slow diffusive exchange between water molecules in junction domains and inside gel meshes. This condition is defined by Eq. (2) (Belton & Hills, 1987; Hills et al., 1990)

Table 1
Proton transverse relaxation times and signal percentages at day 1 after manufacturing (fresh Mozzarella) of the four components in the relaxation curve of Mozzarella di Bufala Campana "Francia", "Taverna Penta", "Rivabianca", "La Baronia" and "Sorì"

Component	Serum water		Fat		Entrapped water		Junctions zones water	
	T_2 (ms)	%	T_2 (ms)	%	T_2 (ms)	%	T_2 (ms)	%
Francia	951 ± 36	28 ± 6	160 ± 15	20 ± 3	47 ± 3	37 ± 1	7.3 ± 0.3	15 ± 4
Taverna penta	1098 ± 70	13 ± 2	178 ± 12	22 ± 1	45 ± 1	36 ± 3	7.9 ± 0.7	29 ± 2
Rivabianca	1005 ± 68	13 ± 2	150 ± 12	21 ± 2	41 ± 3	46 ± 6	8.0 ± 0.5	20 ± 3
La Baronia	1038 ± 90	24 ± 2	166 ± 14	23 ± 2	43 ± 3	34 ± 4	7.9 ± 0.3	19 ± 3
Sorì	998 ± 54	13 ± 4	150 ± 13	22 ± 3	44 ± 2	42 ± 4	7.5 ± 0.5	23 ± 2

Data are the averages of four samples from a single Mozzarella di Bufala Campana cheese loaf (Mozzarella di Bufala Campana "Francia" samples have been prepared by sampling three loaves.) and errors represent the standard deviations of the four values.

$$\frac{D}{a^2} < \left| \frac{1}{T_{2,\mathrm{jw}}} - \frac{1}{T_{2,\mathrm{ew}}} \right| \tag{2}$$

where a is the characteristic dimension of a spatial heterogeneity, D is the water self-diffusion coefficient and $T_{2,\mathrm{jw}}^{-1}$ and $T_{2,\mathrm{ew}}^{-1}$ are the intrinsic relaxation rates of water molecules in junction zones and inside meshes of casein network, respectively. So, assuming that water diffusion in this compartment is the same as that for bulk water, and knowing the D value for water to be, at 8 °C, $1.4 \times 10^{-9}\ \mathrm{m^2\,s^{-1}}$ (Holtz, Heil, & Sacco, 2000), Eq. (2) led to the calculation that water molecule diffusive domains in the casein gel-like network should have sizes larger than 4 μm.

Furthermore, since the ratio D/a^2 is nothing but the diffusive exchange rate constant, k_{diff}, a value of $k_{\mathrm{diff}} \approx 116\ \mathrm{s^{-1}}$ for diffusion of water molecules can be derived.

Now, even though the slow diffusive exchange regime explains the multi-exponential relaxation in the Mozzarella cheese casein network compartment, it does not in itself explain why the relaxation rates of entrapped water and junction zone water are faster than that of bulk water. As far as the measured entrapped water relaxation is concerned, it is essentially controlled by a diffusive exchange from the bulk to the biopolymer interface, which acts as a relaxation surface sink, and surface relaxation will become significant. If relaxation on the surface is fast compared with diffusion to the surface – “diffusion limited relaxation” regime – the observed water transverse relaxation rate is independent of the effective surface relaxation strength and is given by Eq. (3) (Belton et al., 1988).

$$\frac{1}{T_{2,\mathrm{ew}}} = \frac{1}{T_{2,\mathrm{w}}} + \frac{\pi^2 \cdot D}{a^2} \tag{3}$$

where $T_{2,\mathrm{w}}^{-1}$ is the water protons intrinsic relaxation rate. A $T_{2,\mathrm{w}}$ value (2.50 ± 0.01 s) was measured by an independent CPMG experiment on conservation liquid of Mozzarella di Bufala Campana “Francia”. Assuming that water diffusion is in the order of that for bulk water, Eq. (3) gives a maximum diffusive distance (Belton et al., 1988) $a = 26$ μm. This is the maximum distance in that it is calculated assuming that the compartment is bounded by extremely active surface relaxation sinks a μm apart.

An alternative estimate of diffusive domain size is obtained by simply assuming unrestricted diffusion and calculating the average distance that a freely diffusing water molecule travels in a time t equal to $T_{2,\mathrm{ew}}$ according to the Einstein equation (4) (Einstein, 1956)

$$\langle a \rangle = \sqrt{6 \cdot D \cdot t} \tag{4}$$

This estimate gives an average diffusion distance of 20 μm for entrapped water molecules.

The range of spatial heterogeneity for the entrapped water compartment size, calculated by Eqs. (2) and (3), 4 μm < a < 26 μm, is consistent with the mean value of $\langle a \rangle$ derived by the Einstein equation (4).

Now, let us consider the T_2 value of water molecules in junction zones of the casein network, $T_{2,\mathrm{jw}}$, which is dominated by the fast chemical exchange of water protons with exchangeable protons of casein molecules, according to the Eq. (5) (Hills et al., 1989)

$$\frac{1}{T_{2,\mathrm{jw}}} = \frac{1}{T_{2,\mathrm{w}}} + \frac{p_{\mathrm{exch}}}{T_{2,\mathrm{exch}} + k_{\mathrm{exch}}^{-1}} \tag{5}$$

where p_{exch} and $T_{2,\mathrm{exch}}$ are the casein-exchangeable protons molar fraction and transverse relaxation time, respectively; k_{exch} is the pseudo-first-order rate constant of chemical exchange between water and biopolymer.

p_{exch} is given by the number of exchangeable protons in casein and can be estimated to be about 0.02 (Gottwald, Creamer, Hubbard, & Callaghan, 2005), $T_{2,\mathrm{exch}}$ is in the order of a few tens of microseconds, and it can be neglected compared to k_{exch}^{-1} (Hills et al., 1990); then from the measured $T_{2,\mathrm{jw}} = 7.3 \pm 0.3$ ms, a value of $k_{\mathrm{exch}} \approx 10^4\ \mathrm{s^{-1}}$ can be derived. Clearly, this is only a rough estimation by assuming casein to be the unique protein in the sample (it is the predominant Mozzarella protein, ca. 70% of total protein, Guinee, Feeney, Auty, & Fox, 2002) and by neglecting the contribution of $T_{2,\mathrm{exch}}$ in Eq. (5).

The obtained values of diffusive and chemical exchange rate constants, $k_{\mathrm{diff}} \approx 116\ \mathrm{s^{-1}}$ and $k_{\mathrm{exch}} \approx 10^4\ \mathrm{s^{-1}}$, are of the same magnitude order as the values derived by Hills et al. (1990) in a casein micelles suspension (~ 80% w/w). In their study, about the dependence of the water proton transverse relaxation rate on the CPMG sequence pulse spacing (τ_{cp}) at 100 MHz, the authors found a CPMG double dispersion curve with two mid-points, at $1/\tau_{\mathrm{cp}} \approx 1$–$2 \times 10^2\ \mathrm{s^{-1}}$ and 3–$6 \times 10^3\ \mathrm{s^{-1}}$, respectively. From these values, an estimate for diffusive and chemical exchange rate constants was obtained, the points of inflexion in the CPMG dispersion curve occurring when the pulse rate, $1/\tau_{\mathrm{cp}}$, equalled the diffusive and chemical exchange rates, respectively.

3.2. Relaxation measurements in aged Mozzarella di Bufala Campana cheese

Let us first consider T_2 values of serum water, entrapped water, junction zone water and fat droplets as a function of aging time (from day 1 to 14) of Mozzarella di Bufala Campana “Francia” samples (summarised in Table 2). Inspection of the data shown in Table 2 shows a nearly constant value, with aging of Mozzarella, for T_2 transverse relaxation time of entrapped water and junction zone water, indicating that water in the gel-like casein structure, as concerns its microscopic interactions, experiences no substantial differences, at least in the sampled time (days 1–14).

On the contrary, serum water T_2 values show a notable and gradual lowering from day 1 to day 7, until a constant limiting value is obtained. This could be related to changes in micro-structure during storage of Mozzarella cheese. In fact, as reported in the literature (Paulson, McMahon, & Oberg, 1998; McMahon, Fife, & Oberg, 1999; Oommen, McMahon, Oberg, Broadbent, & Strickland, 2002), proteins are not in a quiescent state immediately after stretching

Table 2
Proton transverse relaxation times and signal percentages at the different aging times (days) of the four components in the relaxation curve of Mozzarella di Bufala Campana “Francia”

Component: Days	Serum water		Fat		Entrapped water		Junctions zones water	
	T_2 (ms)	%	T_2 (ms)	%	T_2 (ms)	%	T_2 (ms)	%
1	951 ± 36	28 ± 6	160 ± 15	20 ± 3	47 ± 3	37 ± 1	7.3 ± 0.3	15 ± 4
2	780 ± 32	28 ± 2	149 ± 8	21 ± 1	43 ± 1	35 ± 2	7.4 ± 0.1	17 ± 1
3	764 ± 74	23 ± 6	148 ± 9	22 ± 3	44 ± 2	37 ± 5	7.1 ± 0.2	17 ± 1
4	698 ± 32	27 ± 3	137 ± 7	20 ± 1	40 ± 2	38 ± 2	6.8 ± 0.3	15 ± 2
7	631 ± 31	27 ± 1	122 ± 9	21 ± 1	41 ± 1	38 ± 2	6.4 ± 0.1	14 ± 1
9	637 ± 2	31 ± 3	131 ± 1	23 ± 3	44 ± 1	34 ± 3	6.9 ± 0.1	13 ± 1
11	637 ± 28	34 ± 5	122 ± 4	21 ± 3	43 ± 1	31 ± 2	6.7 ± 0.3	14 ± 6
14	629 ± 25	33 ± 3	123 ± 13	23 ± 2	44 ± 4	32 ± 3	6.6 ± 0.2	12 ± 2

Data are the averages of twelve samples, four from each of the three loaves of Mozzarella di Bufala Campana, and errors represent the standard deviations.

and moulding, but undergo a continuous structural rearrangement. In particular, McMahon et al. (1999) examined Mozzarella cheese micro-structure by scanning electron micrographs and reported that the fat–serum channels appearance continued to change from day 1 through days 7, 14, and 21. The protein matrix expanded into the areas between fat globules, thin strands of protein material, encroaching from all sides, connected the fat–serum channel walls and partially occupied the space previously filled by the interstitial serum between the closely packed fat globules. As a consequence, some interactions between proteins were replaced with interactions of proteins with the bulk phase water molecules – the proteins became more hydrated and their hydrodynamic volumes increased. Clearly, from the NMR point of view, this phenomenon causes serum water T_2 to become lower because serum water molecules are much more likely to get into contact with the channel walls formed by protein fibres. That is also supported by the decrease, even if less pronounced, in fat T_2 values, as shown in Table 2. Hence a correlation of the lowering of serum water T_2 values with the size of fat–serum channels fits the description of McMahon et al. (1999) of the Mozzarella aging during refrigerated (4 °C) storage, because the observed decrease of serum water T_2 value can be interpreted as due to a reduction of fat–serum channel volume, that increases channel walls relaxation effect on water molecules.

Finally, proton transverse relaxation curves of Mozzarella di Bufala Campana samples, produced by different artisan cheese-makers as a function of aging time, were measured. The trend of T_2 values with aging for samples produced by different cheese-makers is the same. Entrapped water and junction zone water T_2 values do not change in the sampled storage time, while serum water T_2 value shows a gradual lowering from day 1 to day 7 until a limiting value is obtained. The serum water T_2 value behaviour, with aging time of Mozzarella di Bufala Campana samples by different cheese-makers, is shown in Fig. 1. As shown, the

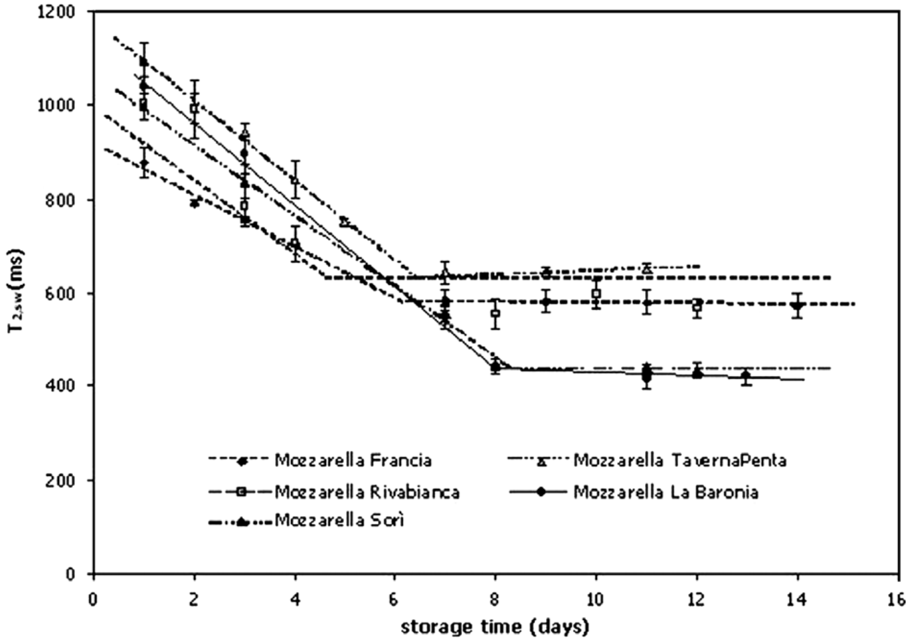

Fig. 1. Changes in serum water T_2 values of Mozzarella di Bufala Campana “Francia” (Caseificio Fratelli Francia), “Taverna Penta” (Azienda Agricola Morese), “Rivabianca” (Cooperativa Allevatori di Bufale di Paestum), “La Baronia” (Caseificio La Baronia) and “Sorì” (Caseificio Sorì Italia) during storage time at 8 °C. Lines are guides to eyes only.

serum water T_2 value lowering, linearly related to structural changes inside the system, can be used to monitor aging of Mozzarella di Bufala Campana cheese during the first seven days after manufacture.

Our results integrate those obtained on similar systems (pasta filata cheese), by other authors who investigated age-related changes in functionality and micro-structure of bovine Mozzarella (Fox, 1989; Guo & Kindstedt, 1995; McMahon et al., 1999; Oommen et al., 2002; Imm et al., 2003). Guo and Kindstedt (1995) found that quantity of expressible serum of Mozzarella cheese decreased dramatically during 16 days of storage, indicating a substantial increase in water-holding capacity. McMahon et al. (1999) reported that, during refrigerated (4 °C) storage (from day 1 to day 21) of home-made bovine Mozzarella, the amount of expressible serum decreased, so that by day 14 very little serum was expressed, while the meltability increased (the largest serum amounts and meltability changes were observed from day 1 to 7, followed by minimal increase from day 7 to 21). Oommen et al. (2002) reported that hardness and stretch quality of Mozzarella cheese decreased with age in 14 days. Imm et al. (2003) reported that substantial increase in meltability, and free oil formation of bovine Mozzarella occurred at 1 week of refrigerated (4 °C) storage. Finally, Romano, Ricciardi, Salzano, and Suzzi (2001) reported 7 days as the shelf life of Mozzarella di Bufala Campana cheese. Our results suggest that an objective and repeatable assessment of freshness of Mozzarella di Bufala Campana may be possible by measuring the serum water T_2 value. It is also notable that visual observations of Mozzarella di Bufala Campana "Francia" during storage time confirmed the structure changes: the first day after manufacture the Mozzarella had a porcelain white colour; its surface appeared tight, smooth and moist (neither too dry nor too wet); the skin was thin (about 1 mm) while the pasta had elastic consistency with small cavities (called "occhiature") and pearls of milk whey seeped out when cutting it. After 3 days, the Mozzarella began loosing consistency and skin became thicker; significant changes were found after 5 or 7 days of storage, when the cheese had a dull white colour and it lost its characteristic structure of overlapping sheets; its consistency became more "buttery", and the small cavities were transformed into an elongated fibrous matrix and liquid separated from the cheese.

In conclusion, despite acknowledging that the relationship among various physical–chemical characteristics and shelf-life (meant as time before cheese considered unsuitable for consumption, because it is still safe but its optimal quality is no longer guaranteed) of Mozzarella is not obvious, it seems to be very relevant to have identified a reliable physical–chemical parameter – the serum water T_2 – whose value is useful for monitoring evolution of Mozzarella samples. The definition of cheese characteristics by an analytical procedure, such a low-resolution NMR protocol, could be important for establishing the quality, typicality or specificity of the products. Thus, our findings may be considered of some importance because Mozzarella di Bufala Campana cheese is a high grade Italian cheese, as certified by the PDO trademark: its freshness is fundamental for fully appreciating the Mozzarella taste (Coppola, Villani, Coppola, & Parente, 1990; Gambera & Surra, 2003).

4. Conclusion

Abundant water protons may be considered as sensitive probes useful for describing, not only cheese morphology, but also easily monitoring a cheese system. Transverse magnetization decay curves of Mozzarella di Bufala Campana samples, analysed on the basis of the diffusive and chemical exchange model, gave information on water diffusive domain size, as well as on water molecules dynamics. Particularly, the observed serum water T_2 value decrease with storage time can be used to monitor aging of Mozzarella samples, at least up to seven days after manufacture. Finally, it has to be pointed out that low-resolution NMR measurements do not require any pre-treatment of the sample to be investigated and are very rapid. The NMR measurement, in fact, takes only few minutes (4–5 min to collect the CPMG curve and 1–2 min for the fitting procedure). So, once developed, the measuring protocol, based on the use of low-resolution NMR equipment, may be used for firmness and quality control applications.

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